Polyhedron 38 (2012) 1–6

Contents lists available at SciVerse ScienceDirect

Polyhedron

journal homepage: www.elsevier.com/locate/poly

A new potential contrast agent for magnetic resonance imaging: Synthesis and relaxivity studies of a gadolinium(III) complex of glucose-6-phosphate conjugated 1,4,7,10-tetraazacyclododecane-1,4,7-triacetic acid

S. James Ratnakar, N. Arockia Samy, V. Alexander *

Department of Chemistry, Loyola College, Chennai 600034, India

ARTICLE INFO

Article history:
Received 12 December 2011
Accepted 2 February 2012
Available online 8 March 2012

Keywords:
MRI
Gadolinium complexes
Contrast agents
Relaxivity
Glucose-6-phosphate
Polyazacarboxylate ligands
Lanthanide complexes

ABSTRACT

Synthesis and longitudinal and transverse relaxivities of a gadolinium(III) complex, [Gd(DO3A-Pr-Glu-6-phos)$(H_2O)_2$] (**4**), of glucose-6-phosphate conjugated DO3A (DO3A-Pr-Glu-6-phos = 10-(3-(glucose-6-phosphate)oxypropyl)-1,4,7-tris(carboxymethyl)-1,4,7,10-tetraazacyclododecane and DO3A = 1,4,7,10-tetraazacyclododecane-1,4,7-triacetic acid) are reported. DO3A-Pr-Glu-6-phos (**L**[1]) is synthesized by the reaction of bromopropane appended DO3A (**3**) with glucose-6-phosphate at room temperature. The magnetic moment of **4** is 7.49 BM, which is close to that of the free gadolinium(III) ion. The X- and Q-band epr spectra of **4** at LNT show a broad band with *g*-values of 2.167 and 2.033, respectively. The higher longitudinal relaxivity of **4** (r_{1p} = 6.99 mM^{-1} s^{-1}, 24 MHz, 35 °C ± 0.1) than that of $[Gd(DOTA)(H_2O)]^-$ (r_{1p} = 3.56 mM^{-1} s^{-1}, 20 MHz, 39 °C, pH 7.3) and [Gd(DO3A)$(H_2O)_2$] (r_{1p} = 4.8 mM^{-1} s^{-1}, 20 MHz, 40 °C) is attributed to the nature of the glucose-6-phosphate pendant arm. The longitudinal relaxivity of the complex in the presence of β-cyclodextrin increases to 9.62 mM^{-1} s^{-1} due to the formation of the inclusion complex. The transverse relaxivity of **4** is 7.02 mM^{-1} s^{-1} and the r_{2p}/r_{1p} ratio of 1.01 indicates that it is a T_1-weighted contrast agent.

1. Introduction

The development of magnetic resonance imaging (MRI) as a clinical modality has provoked an explosive growth of interest in the study of gadolinium(III) complexes as contrast enhancing agents (CAs) for MRI [1–3]. This technique relies upon the acquisition of the images of soft tissues which are the topological representation of in vivo water protons. The image contrast is based on the differences in the proton relaxation rates of water molecules in different tissues. Contrast agents enhance the intrinsic contrast of the magnetic resonance images by accelerating the relaxation rates of water protons. Gadolinium(III) complexes of polyazacarboxylate ligands have been extensively studied as CAs and the topic has been reviewed [4–10]. New classes of MRI contrast agents [11,12] such as blood pool agents [13–17], targeting CAs [11] and smart contrast agents such as those that are pH sensitive [18–22], oxygen pressure (pO_2) responsive [23,24], enzyme responsive [25–27] and metal ion sensitive [28–31] have been reported. Receptor induced magnetization enhancement (RIME) CAs involving targeting Gd(III) chelates to protein binding sites [32,33], chemical exchange saturation transfer (CEST) CAs [34–37] and LIPOCEST agents for improved sensitivity [38] have also been reported.

The FDA approved CAs are low molecular weight gadolinium(III) chelates which exhibit poor bioselectivity for tissues and organs. They are cleared rapidly from the blood stream and a higher dosage is required for prolonged clinical examinations. There is an ongoing demand for the development of highly efficient pathology specific and organ specific CAs. In general, in the case of gadolinium(III)-based CAs, higher relaxivity may be achieved by improving the inner-sphere relaxivity which, according to SBM theory [39], depends primarily on the number of coordinated water molecules (q) and the correlation time (τ_c). The correlation time τ_c, which modulates the dipole–dipole relaxation mechanism, depends on the molecular rotational correlation time (τ_R) of the complex, the exchange rate ($k_{ex} = 1/\tau_m$) of the coordinated water molecules and the electronic relaxation time ($T_{1e,2e}$) of the metal ion. One important strategy to attain high relaxivity is by slowing down the molecular rotation by increasing the molecular weight and dimension by binding gadolinium(III) chelates to systems of different dimensions [1,2].

Biomolecules-conjugated gadolinium(III) chelates are expected to be biocompatible and increase relaxivity by slowing down the molecular rotation in solution. Examples of biomolecule- and biomacromolecule conjugated gadolinium(III) chelates include a gadolinium(III) chelate of galactopyranose functionalized DO3A, which

* Corresponding author. Tel.: +91 44 28175351; fax: +91 44 28175566.
E-mail address: valexander@rediffmail.com (V. Alexander).

0277-5387/$ - see front matter © 2012 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.poly.2012.02.022

exhibits selective activation from low to high relaxivity (r_{1p} = 0.903–2.72 mM^{-1} s^{-1}) with respect to the enzymatic response by Meade and co-workers [25], a Gd(DO3A) chelate appended onto a polysaccharide backbone [starch-(Gd-DO3A)] (r_{1p} = 14.1 ± 0.1 mM^{-1} s^{-1} and r_{2p} = 17.8 ± 0.9 mM^{-1} s^{-1}, 20 MHz, 39 °C) by Brasch and co-workers [40], a Gd(DO3A)-functionalized macromolecular conjugate of dextran [CMD-A_2-Gd-(DO3A)] (r_{1p} = 10.59 mM^{-1} s^{-1}, 37 °C) as an intravascular CA for myocardial perfusion by Corot et al. [41], DO3A conjugated cholesterol for adrenal imaging by Muhler et al. [42], a gadolinium(III) chelate of glucitol conjugated DO3A (r_{1p} = 5.19 mM^{-1} s^{-1}) by Aime et al. [43], and the gadolinium(III) complex of ATP-conjugated DO3A [Gd(DO3A-Pr-ATP)(H_2O)$_2$] (r_{1p} = 6.51 and 5.64 mM^{-1} s^{-1} at pH 5.6 and 8.4, respectively, and r_{2p} = 7.48 mM^{-1} s^{-1}, 24 MHz, 35 ± 0.1 °C) [44]. Gadolinium(III) complexes of phosphonic acid and phosphinate based pendant arms have also been reported [45–51]. We report herein the synthesis and relaxivity studies of a gadolinium(III) chelate of glucose-6-phosphate conjugated DO3A (**L**[1]) (Chart 1).

2. Experimental

2.1. Chemicals

Triethylenetetraamine (99%), *p*-toluenesulfonyl chloride, 1,2-dibromoethane, 1,3-dibromopropane, chloroacetic acid, xylenol orange, β-cyclodextrin, Celite, potassium carbonate anhydrous and Tris buffer (E. Merk, India) were used as received. Glucose-6-phosphate monosodium salt (Aldrich) was converted into the free form by passing it through Amberlite IR120 (H^+, strongly acidic) (Aldrich) cation exchange resin and eluting with triply distilled water. The resin was washed with distilled water five times before use. Triethylamine and perchloric acid (Merck, India); sodium hydroxide and potassium hydroxide pellets (Rankem, India); gadolinium(III) carbonate (Indian Rare Earths Ltd.) were used as received. Gadolinium(III) perchlorate hydrate was prepared from gadolinium(III) carbonate and perchloric acid in water and recrystallized from triply distilled water prior to use. Cyclen was synthesized by the method of Chavez and Sherry [52]. The solvents were purified by standard procedures [53]. HPLC grade water was used for the relaxivity studies. DO3A and bromopropane appended DO3A were synthesized by the method reported by us [44].

2.2. Physical measurements

Infrared spectra were recorded on a Perkin-Elmer Spectrum RX-I FT-IR spectrometer in the range 4000–400 cm^{-1} using KBr pellets. Potassium bromide (FT IR grade, Aldrich) was used to make the pellets. FAB mass spectra were recorded on a Jeol SX-102/DA 6000 mass spectrometer/data system using argon (6 kV, 10 mA) as the FAB gas. The accelerating voltage was 10 kV and the spectra were recorded at room temperature using *m*-nitrobenzyl alcohol as the matrix. Electrospray ionization mass spectra were recorded using a Micromass Quattro-II Triple Quadrupole mass spectrometer. The sample was dissolved in water and introduced into the ESI capillary using a 5 μL syringe pump. The ESI capillary was set at 3.5 kV with a cone voltage of 40 V. CHN microanalyses were carried out using a Perkin-Elmer 2400 Series II CHNS/O Elemental Analyzer interfaced with a Perkin-Elmer AD 6 Autobalance. Helium (analytical grade) was used as the carrier gas. Analytical and preparative HPLC analyses were carried out using a Varian PrepStar 218 (Varian Instruments Inc., USA) binary gradient solvent delivery module with an inline three channel degasser Model 2000 for solvent delivery. A Rheodyne injector valve (20 μL) was used for the sample injection. HPLC column (250 × 4.6 × 1/4″ Valco, Microsorb-MV 100-5 C18, analytical) was used for the HPLC analysis of the free ligand and its gadolinium(III) complex. A UV–Vis detector (Model 345) operating in the range 190–1100 nm was used. The fraction was collected using a Model 704 fraction collector. EPR spectra were recorded on a Jeol instrument at the Q-band (34.5 MHz) and the X-band (9.4 MHz) with a scan range of 8 kG and the field was set at 12 500 T. Magnetic susceptibility measurements were carried out on an EG&G PAR Model 155 vibrating sample magnetometer at 25 °C. ^1H and ^{13}C NMR spectra were recorded in D_2O and $CDCl_3$ (99.95 atom % D, Aldrich) on a Jeol GSX-400 multinuclear NMR spectrometer working at 400 MHz (for ^1H) and at 100 MHz (for ^{13}C) at 25 °C.

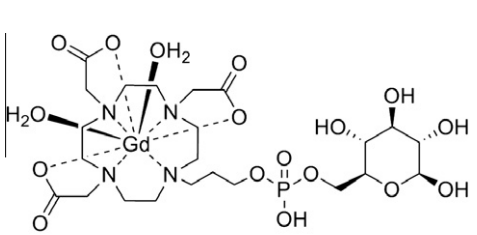

Chart 1. Structure of the gadolinium(III) complex of 10-(3-(glucose-6-phosphate)oxypropyl)-1,4,7-tris(carboxymethyl)-1,4,7,10-tetraazacyclododecane.

2.3. Longitudinal relaxivity (r_{1p}) measurements

The longitudinal relaxivity of the gadolinium(III) complex was determined from the spin lattice relaxation time (T_1). The T_1 measurements were carried out on a Maran wide line NMR (Resonance Instruments Ltd., UK) operating at 24 MHz and 35 ± 0.1 °C. The temperature was controlled using a temperature console. The solutions of the complex were taken in a 10 mm stoppered quartz tube and the instrument parameters were optimized for each T_1 measurement. Solutions of five concentrations of the complex were prepared in HPLC grade water (Merck, India) in a 5 mL standard measuring flask (Vensil, Class "A"). The presence of the free gadolinium(III) ion in the solution has been checked by the xylenol orange test. The T_1 measurements were made using the standard inversion recovery pulse sequence (180°-τ-90°) with phase sensitive detection [54], with τ values ranging from 50 μs to 6 s for each concentration of the complex. The computer program WINFIT was used to plot the time versus signal intensity to get an exponential plot, and the T_1 values were calculated from the plot. A plot of $1/T_1$ versus concentration of the complex gave a straight line and the slope was taken as the longitudinal relaxivity (r_{1p}), which was normalized to 1 mM concentration of the complex. The longitudinal relaxivity at pH 8.5 was measured by adding Tris buffer. The longitudinal relaxivity in the presence of β-cyclodextrin was determined by adding an aqueous solution of β-cyclodextrin and keeping the solution overnight at ambient temperature to attain equilibrium. The concentration of β-cyclodextrin was maintained 50 times higher than that of the complex to ensure the complete formation of the inclusion complex.

2.4. Transverse relaxivity (r_{2p}) measurements

The transverse relaxivity was determined from the spin–spin relaxation time (T_2) under the same experimental conditions used to determine longitudinal relaxivity. A standard CPMG (Carr–Purcell–Meiboom–Gill) pulse sequence (90°-τ-180°) [55] with a τ value of 50 μs was used to determine T_2. The computer program WINFIT was used to plot the time versus signal intensity to get an exponential curve, and T_2 was calculated from the graph. The T_2 values for five different concentrations of the complex